Image Shift Correction in Microscopic Time Lapse Sequences

1. General Information

It is of importance to ensure mechanical stability of your live cell imaging set-up during a time lapse experiment. Vibration effects or the tolerance in the place recovery of the motorized stage can cause a (slight) shift of the image sequence. Every external movement can overlay the observed cell motion. This is of particular importance when using higher magnifications or when working with slow migrating cells, as the distance being covered by each cell is comparably small. This Application Note describes a method to calculate the externally caused pixel shift which is then subtracted from the cell track coordinates. The resulting data summarizes the actual cell migration parameters.

2. Application Example

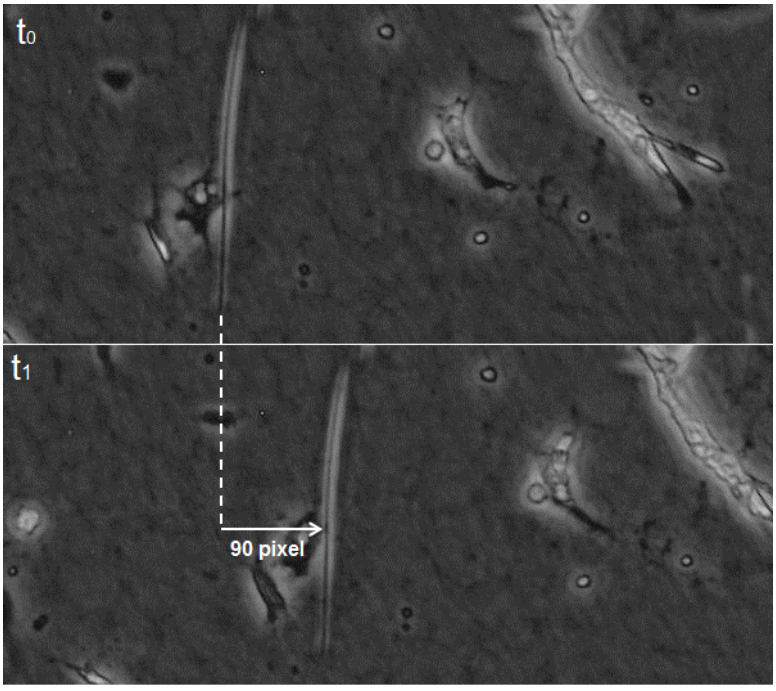


Figure 1 A displacement of the stage top incubator created a permanent pixel shift of about 90 pixel during the experiment.

Figure 1 shows as one example the need for the correction of a pixel shift in an image sequence. A displacement of the stage top incubator created a permanent pixel shift of about 90 pixel while performing the experiment. Plotting the cell tracks (Figure 2) and analysing the migration parameters (Table 1) gave the false impression of a directed cell migration. However, including the pixel shift in the data analysis revealed that the apparently present directed cell migration was only created by the pixel shift.

The given method is not only applicable to compensate one large pixel shift, but can also be used for small shifts which can occur throughout the whole measurement.

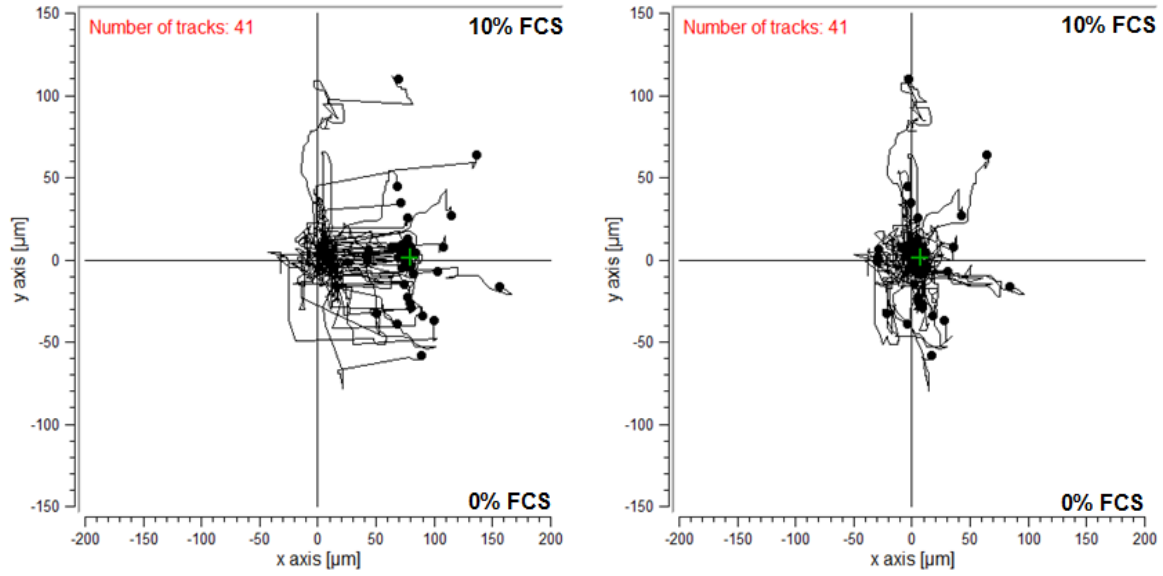


Figure 2 Trajectory plot of 40 cells in the time period from 0 to 24 hours. The left plot shows the uncorrected data, the right plot the corrected data.

Table 1 Comparison of the migration parameters of the uncorrected and the corrected data. The obtained values for the uncorrected tracks indicate a directed cell migration, even though this is only attributed to the existing pixel shift.

Parameter		Without Correction	With Correction
FMI	X	0.40	0.04
	Y	0.01	0.02
Directness		0.41	0.14
Center of mass [µm]	X	78.79	6.79
	Y	1.71	1.71
Rayleigh Test [p-value]		1.08e-15	0.04
Mean accumulated distance [µm]		223	176
Mean Euclidean distance [µm]		83	26
Mean velocity [µm/min]		0.15	0.12

3. Principle

Several working steps are needed for the correction of pixel shifts during an image sequence:

1. **Track between 30 and 40 cells of each time lapse measurement.** We recommend, for example, the ImageJ plugin, Manual Tracking. This plugin is able to quantify the movement of objects between frames of a temporal stack.

2. **A reference track is needed for each chemotaxis chamber**. A rigid point, such as a defined position of the channel border, has to be tracked over the whole image sequence. When using a collagen fiber as rigid point please make sure that no fiber movement occurs due to tensional forces created by cell movement. The first image of the reference track serves as comparative value ($X_{ref,1}$ *and* Y_{ref1}). The pixel shift ($X_{shift,i}$ *and* $Y_{shift,i}$) between all subsequent images ($X_{ref,i}$ *and* $Y_{ref,i}$) and the comparative value has to be calculated.

$$X_{shift,i} = X_{ref,i} - X_{ref,1}$$

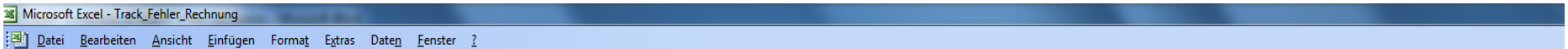
$$Y_{shift,i} = Y_{ref,i} - Y_{ref,1}$$

3. **Correction of your cell tracks.** The obtained track coordinates (X_i *and* Y_i) of each image have to be corrected by the calculated pixel shifts. This is performed by subtracting the pixel shift from the raw data cell track coordinates. To do this, it is necessary to duplicate the cell track data and to paste the reference track data in the same Excel file (Figure 3).

$$X_{corr} = X_i - X_{shift,i}$$

$$Y_{corr} = Y_i - Y_{shift,i}$$

4. **Copy the corrected values into a new Excel file.** This file should be saved as excel (.xls or .xlsx) and as text document (.txt). The exel document allows subsequent changes while the text document can be imported into the Chemotaxis and Migration Tool (Free software provided by ibidi) for plotting your data and analyzing the chemotactical effect.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1		Raw data									Corrected values									Reference Track		
2																						
3		Track n°	Slice n°	X	Y	Distance	Velocity	Pixel Value			Track n°	Slice n°	X_{corr}	Y_{corr}	Distance	Velocity	Pixel Value		X_{ref}	Y_{ref}	$X_{ref,i} - X_{ref,1}$	$Yref,i - Y_{ref,1}$
4	1	1	1	1586	928	-1	-1	149		1	1	1	1586	928	-1	-1	149		1210	1122	0	0
5	2	1	2	1586	928	0	0	155		2	1	2	1586	928	0	0	155		1210	1122	0	0
6	3	1	3	1586	928	0	0	143		3	1	3	1586	928	0	0	143		1210	1122	0	0
7	4	1	4	1586	928	0	0	142		4	1	4	1586	928	0	0	142		1210	1122	0	0
8	5	1	5	1586	928	0	0	180		5	1	5	1584	928	0	0	180		1212	1122	2	0
9	6	1	6	1590	932	0.73	0.365	175		6	1	6	1588	932	0.73	0.365	175		1212	1122	2	0
10	7	1	7	1590	932	0	0	141		7	1	7	1588	932	0	0	141		1212	1122	2	0
11	8	1	8	1590	932	0	0	142		8	1	8	1588	932	0	0	142		1212	1122	2	0
12	9	1	9	1590	932	0	0	160		9	1	9	1588	932	0	0	160		1212	1122	2	0
13	10	1	10	1590	932	0	0	168		10	1	10	1588	932	0	0	168		1212	1122	2	0
14	11	1	11	1590	932	0	0	149		11	1	11	1588	932	0	0	149		1212	1122	2	0
15	12	1	12	1590	932	0	0	147		12	1	12	1588	932	0	0	147		1212	1122	2	0
16	13	1	13	1594	932	0.516	0.258	141		13	1	13	1592	932	0.516	0.258	141		1212	1122	2	0
17	14	1	14	1594	934	0.258	0.129	80		14	1	14	1592	934	0.258	0.129	80		1212	1122	2	0
18	15	1	15	1594	934	0	0	136		15	1	15	1592	934	0	0	136		1212	1122	2	0
19	16	1	16	1594	934	0	0	143		16	1	16	1592	934	0	0	143		1212	1122	2	0
20	17	1	17	1594	934	0	0	155		17	1	17	1592	934	0	0	155		1212	1122	2	0
21	18	1	18	1594	934	0	0	112		18	1	18	1592	934	0	0	112		1212	1122	2	0
22	19	1	19	1594	934	0	0	64		19	1	19	1592	934	0	0	64		1212	1122	2	0
23	20	1	20	1594	934	0	0	102		20	1	20	1592	934	0	0	102		1212	1122	2	0
24	21	1	21	1594	934	0	0	83		21	1	21	1592	934	0	0	83		1212	1122	2	0
25	22	1	22	1594	934	0	0	112		22	1	22	1592	934	0	0	112		1212	1122	2	0
26	23	1	23	1594	934	0	0	82		23	1	23	1592	934	0	0	82		1212	1122	2	0
27	24	1	24	1594	934	0	0	92		24	1	24	1592	934	0	0	92		1212	1122	2	0
28	25	1	25	1592	934	0.258	0.129	126		25	1	25	1590	934	0.258	0.129	126		1212	1122	2	0
29	26	1	26	1592	934	0	0	164		26	1	26	1590	934	0	0	164		1212	1122	2	0
30	27	1	27	1592	934	0	0	173		27	1	27	1590	934	0	0	173		1212	1122	2	0
31	28	1	28	1592	934	0	0	155		28	1	28	1590	934	0	0	155		1212	1122	2	0
32	29	1	29	1592	934	0	0	169		29	1	29	1590	934	0	0	169		1212	1122	2	0
33	30	1	30	1592	934	0	0	173		30	1	30	1590	934	0	0	173		1212	1122	2	0
34	31	1	31	1592	934	0	0	149		31	1	31	1590	934	0	0	149		1212	1122	2	0
35	32	1	32	1592	934	0	0	133		32	1	32	1590	934	0	0	133		1212	1122	2	0
36	33	1	33	1592	934	0	0	158		33	1	33	1590	934	0	0	158		1212	1122	2	0
37	34	1	34	1592	934	0	0	169		34	1	34	1588	936	0	0	169		1214	1120	4	-2
38	35	1	35	1592	934	0	0	180		35	1	35	1584	936	0	0	180		1218	1120	8	-2

Figure 3 Example calculation for the correction of small pixel shifts in image sequences. The pixel shift for each reference picture was calculated and subtracted from the raw data to obtain the corrected track coordinates.

5. **Check the obtained values for correctness.** Open the original as well as the corrected track file with the Chemotaxis and Migration tool to check whether you only corrected your data and did not, by mistake, create an artificial cell movement (Figure 4). The artificial cell movement shown in figure 4 was attributed to a delayed reaction to the pixel shift while tracking the cell. This delayed reaction, in comparison to the reference track, cannot be compensated by this method.

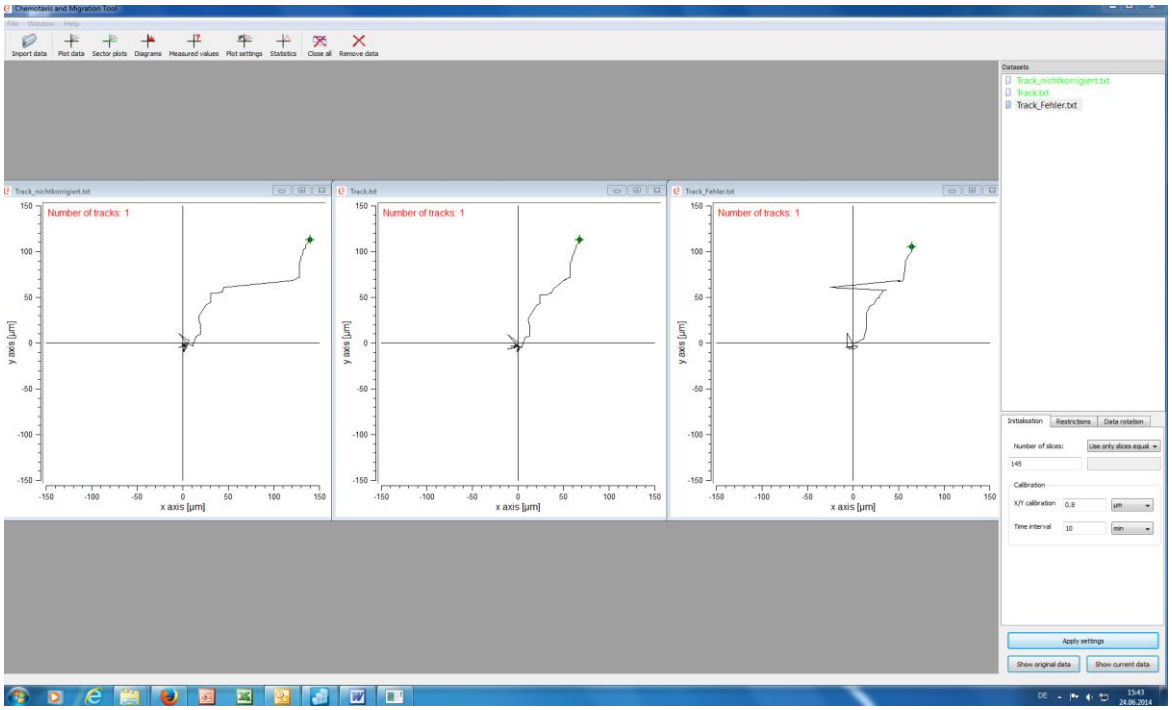


Figure 4 Testing for correction of your data is important. The left plot shows the original data without correction, the middle plot the pixel shift corrected data and the right plot an artificially created cell movement.